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Bio-Efficacy of Cotyledon Oil Extracts and Powders of Melon, *Citrillus Vulgaris* Schrad against Maize Weevils, *Sitophilus Zeamais* Motschulsky (Coleoptera: Curculionidae)

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Research article

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Abstract

Toxicity of cotyledon oil and powder of Melon, *Citrullus vulgaris* Schrad against *Sitophilus zeamais* (Motschulsky) was assessed. Cotyledon oil extract of *C. vulgaris* was mixed with 20 g of maize grains at the concentration rate of 0.5, 1.0, 1.5 and 2.0 % v/w while the cotyledon powder was mixed at the dosage rate of 1.0, 2.0, 3.0 and 4.0 g per 20 g of maize grains. They were all infested with five newly emerged *S. zeamais*. Untreated experiment was set up as control for all the treatments. All treatments were replicated four times and set up in a Complete Randomized Design. The experiments were observed for 192 h to record mortality. After 192 h the experiments were allowed to stay for another one week after which the grains were stained in gentian violet to reveal egg plugs. Thereafter, the experiments were allowed to stay for another 7 weeks to observe adult emergence. Percentage grain damage and weight loss were calculated after 50 days. Cotyledon powders and oils of *C. vulgaris* significantly reduced mortality, oviposition and adult emergence. Mortality of treated experiment was 100 % at the concentration rate of 1.0, 1.5 and 2.0 % v/w by 192 h of the experiment while the mortality of powder treated experiment was 100 % at the treatment level of 3.0 and 4.0 g per 20 g of maize grains. Also, oviposition was 0 % at oil extract concentration level of 1.5 and 2.0 % v/w. The oil and powder significantly reduced grain damage.

Keywords: Toxicity; *Citrullus vulgaris; Sitophilus zeamais;* Mortality; Oviposition

Introduction

Maize (*Zea mays* L.) is among the grains that suffer from serious insect attack during storage especially in the tropical and the sub-tropical parts of the world. It is heavily damaged by maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera; Curculionidae) which is a serious primary pest of maize in the tropics and other

warm regions of the world. It is secondarily found attacking other crops such as rice, guinea corn, yam products, ground nut, cowpea, millet, cassava flour, cocoyam and beniseed in Nigeria [1,2]. Adult weevils and larva feed on wholesome grains and reduce them to powdery form. The developmental activities of the grains often lead to powdering and tainting with their excrements. The larva is the most destructive stage of this weevil [3].

Various methods have been employed in controlling maize weevils both in the field and in storage in the past and the most common one is the use of conventional insecticides which is associated with some shortcomings such as high mammalian toxicity, high level of persistence in the environment, workers safety, insect resistance, ozone layer depletion and health hazard [4-7]. This situation has necessitated the search for acceptable methods of protecting stored products. As such, current research efforts are being focused more on ecologically tolerable control measures. These include the use of inert materials, plant powders, oils and extracts. There is also an increasing awareness that plants possess chemicals which naturally protect them from pests and pathogen. The tropical region is well endowed with wide array of these floristic species with defensive phyto-chemicals and quite a number of them have been used traditionally in protecting maize against weevil attack.

In recent times, some interesting developments have been made in the use of oil and plant extracts because they are biodegradable, and as a result pose fewer problems on the environment [8,9]. Oil of groundnut, *Arachis hypogea* has been reported to effectively control *S. zeamais* infestation [10]. Seed powders of black pepper, *Piper nigum*, *P. guineese*, *P. unbellatum* and *Capsicum frutescens* have been reported to cause high mortality of maize weevil [11-14]. Lajide, *et al*. [13] reported that pulverisd seeds of *Uvaria afzelli*, *Eugenia aromatica* and the bark of *Erythrophleum guineese* were highly toxic to maize weevil when used to surface treat maize grains infested with the weevil. Extracts of pepper fruits, *Dennettia tripetala* have been reported effective against *S. zeamais* [15]. Odeyemi [16] also reported the control of Khapara beetle, *Trogoderma granarium* with vegetable oils. Essential oils and individual compounds from medicinal and aromatics plants have been known to exhibit anti-feedant properties against number of insects [5]. Yahaya, *et al*. [17] reported the effectiveness of some selected seed oils against the fecundity of *Callosobruchus maculatus.*

The present study evaluated the effectiveness of cotyledon powder and oil extracts of melon, *Citrullus vulgaris* against maize weevil, *Sitophilus zeamais.*

Materials and Methods

Rearing of *Sitophilus zeamais*

The parent stock of *S. zeamas* used for this study was obtained from naturally infested maize grains at Iworoko market, Iworoko-Ekiti, Nigeria. The weevils were reared on maize grains in jars covered with muslin cloth held in place with rubber band to allow for gaseous exchange at 27 degree centigrade and 70 % RH. Newly emerged first filial (F_1) generation adult (1:1 male/female ratio) were used. The sexes of *S. zeamais* were determined by examining the snouts with hand lens. The snouts of the male are longer and thinner while that of the female are shorter and fatter. Also, the females have smooth textured bodies while that of the female are rough [18].

Collection and Preparation of Cotyledon Powder of *C. vulgaris*

The unshelled seeds of melon, *C. vulgaris* were purchased from Mojere market, Ado Ekiti, Ekiti state, Nigeria. The seeds were shelled manually and the cotyledons were removed. The cotyledon were air-dried in the laboratory for 10 days after which they were ground into powder, using a Binatone electric grinder Model.

Preparation of Cotyledon Oil Extracts of *C. vulgaris*

The cotyledon extract was prepared by soaking the powder in 70 % ethanol in a plastic container with tight-fitted lid. After 3 days the solution was filtered with muslin cloth. The filtrate was concentrated in a rotary evaporator. The extract obtained was exposed to a slow-blowing fan to get rid of any trace of solvent in it. Extract obtained was poured into a dark bottle and stored in a refrigerator at 4 degree centigrade for subsequent use.

Toxicity Bioassays

Fifty gram of maize grains was measured into 5 cm diameter plastic containers. The cotyledon powders of *C. vulgaris* were applied to the different containers containing the 50 g of maize grains at the doses of 1.0, 2.0, 3.0 and 4.0 g. The containers were then shaken vigorously for uniform spreading on the grains surfaces. The control experiment is without cotyledon powder of *C. vulgaris.*

Different concentrations (0.5, 1.0, 1.5 and 2.0 %) of ethanol oil extracts of *C. vulgaris* were prepared by adding 0.05, 0.1, 0.15 and 0.2 g of the extracts to 10 ml of ethanol respectively. Thereafter 1.0 ml of each concentration was applied to each replicate and thoroughly mixed with the grains with the aid of a glass rod. The containers were left opened and exposed to slow-blowing fan for about 10 min to allow traces of ethanol to escape. A control experiment without any extract was also set up.

Newly emerged Five male and five female were introduced into each of the container containing 0, 0.5, 1.0, 1.5 and 2.0 % v/w cotyledon extracts and that of 1.0, 2.0, 3.0 and 4.0 g of cotyledon powder. Each treatment and the control were replicated four times in a Complete Randomized Experimental Design. The containers were covered with muslin cloth held tightly in place with rubber band. Thereafter, the experiments were allowed to stay for 8 days (192 h) during which the number of dead insects were counted and recorded at 48 h interval. This was done by gently probing the insect with a sharp pin on the abdomen. Insect that did not respond to the probe were considered dead.

Oviposition Bioassays

All insects, both live and dead were removed after 192 h and 30 grains of maize were removed at random from each of the containers containing the powders extracts and the control experiment. The grains were stained in gentian violet to reveal the egg plugs which were counted and recorded.

Emergence of First Filial Generation (F1)

After 7 weeks of introduction of adult maize weevils into the container, the newly emerged adult weevils were counted and recorded.

Percentage Grain Damage

Percentage grain damage was calculated on the 50th day after the introduction of adult weevil into each experimental container by counting the grains from each container. The numbers of perforated grains were recorded and the formula below was used to determine the percentage grain damage.

$$\% \text{ Grain Damage} = \frac{\text{No of perforated grains}}{\text{Total no of grains counted}}$$

Percentage Weight Loss

The initial weight of the grains in each container was taken before the introduction of the weevils. The final weight was then taken at the end of the experiment. Percentage weight was calculated using the formula below:

$$\% \text{ weight loss} = \frac{\text{Initial weight-final weight}}{\text{Initial weight}}$$

Data Analysis

Data were subjected to analysis of variance and where significant differences existed, treated means were separated, using the New Duncan's Multiple Range Test.

Results

Effect of *C. Vulgaris* Cotyledon Extracts on Mortality of *S. Zeamais.*

Mortality of *S. zeamais* increased with increased in concentration of the extracts as revealed by Table 1. Total weevil mortality was recorded with 1.0, 1.5 and 2.0 % w/v by 192 h of exposure to the extracts. The control experiment revealed just 5.0 % mortality by 192 h of exposure, showing that no insect died in some of the replicates out of the ten insects introduced. Also, 76.67 % mortality was recorded with the concentration of 0.5 % v/w.

Effect of *C. Vulgaris* Cotyledon Powder on Mortality of *S. Zeamais.*

Mortality of *S. zeamais* increased with increased in dosage of the powders and the time of exposure of the insects to the powders. All dosages of the powders significantly affected mortality of the weevils (Table 2). There was 100 % mortality with the dosages level of 3.0 and 4.0 g while 80.20 and 65.22 % mortality was recorded with the dosages level of 1.0 and 2.0 g respectively. Only 5 % mortality was recorded in the control experiment.

Effect of *C. Vulgaris* Cotyledon Extracts on Oviposition and Adult Emergence of *S. Zeamais*

All the cotyledon extracts significantly reduced the number of eggs laid by the maize weevil compared to the untreated grains (Table 3) There was no significant different in the number of eggs laid by the insects exposed

to 1.5 and 2.0 % v/w extract concentration The highest number of eggs (110.3) were laid in the control experiment. The percentage adult emergence in the untreated grain was significantly different (P>0.05) from the treated grains and it follows similar trend with the number of egg hatched by the insects exposed to 1.5 and 2.0 % v/w oil extract concentrations. Just 20.2 and 10.22 adults emerged in the concentration of 0.5.0 and 1.0 % v/w respectively.

Percentage mortality at hours post-treatment				
Conc (% w/v)	**48 h**	**96 h**	**144 h**	**192h**
0.5	42.32±1.92b	55.12±3.24b	65.33±2.96b	76.67±2.34b
1.0	50.00±4.42b	62.00±1.25b	90.00±3.24c	100.00±0.00c
1.5	70.00±3.24c	85.00±1.22c	100.00±0.00d	100.00±0.00c
2.0	90.00±0.00d	100.00±0.05d	100.00±0.05d	100.00±0.05c
0.0	0.000±0.00a	0.00±0.00a	5.00±1.64a	5.00±2.00a

Table 1: Mortality of S. zeamais exposed to cotyledon oil extracts of C. vulgaris 3.0. Results.
Means in the same column followed by the same letter(s) are not significantly different.

Percentage mortality at hour post-treatment				
Dosage/50g	**48 h**	**96 h**	**144 h**	**192 h**
1.0	38.33±1.24b	43.32±1.90b	50.00±2.50b	65.22±2.96a
2.0	50.00±3.30c	66.12±4.12c	72.33±1.40c	80.20±1.24c
3.0	72.24±1.14d	90.22±1.33d	100.00±0.00d	100.00±0.00d
4.0	85.67±2.96d	100.00±0.00a	100.00±0.00d	100.00±0.00d
0.0	0.00±0,00a	0.00±0.00a	5.00±1.64a	5.00±2.00b

Table 2: Mortality of *S. zeamais* exposed to cotyledon powders of *Citrullus vulgaris.*
Means in the same column followed by the same letter(s) are not significantly different.

conc (% w/v)	Number of egg Laid	Number of % Egg Hatched
0.5	48.12±1.25c	20.12±1.10c
1.0	30.34±3.12b	10.22±1.50b
1.5	26.22±2.10b	0.00±0.00a
2.0	15.20±1.20a	0.00±0.00a
0.0	110.30±3.25d	88.24±2.20d

Table 3: Effect of *Citrullus vulgaris* oil extracts on oviposition by maize weevil and percentage adult emergence.
Means in the same column followed by the same letter(s) are not significantly deficient.

Dosage/20 g maize	Number of egg laid	% egg hatched
1.0	42.10±3.12b	15.30±1.40b
2.0	22.12±1.30a	10.12±2.20b
3.0	18.20±3.35a	0.00±0.00a
4.0	15.34±1.33a	0.00±0.00a
0.0	108.24±2.20c	90.70±0.33c

Table 4: Effect of *C. vulgaris* cotyledon powders on oviposition by maize weevil adult Emergence (F1 generation).
Means in the same column followed by the same letter(s) are not significantly different.

Effect of *C Vulgaris* Cotyledon Powder on Oviposition and Adult Emergence of *S. Zeamais*

The powders at all dosages significantly reduced oviposition and adult emergence by the weevils compared to the control experiment (Table 4). There was no significant difference in the no of eggs laid by the insects exposed to 2.0, 3.0 and 4.0 g dosage levels. No adult emerged at the dosage levels of 3.0 and 4.0 g cotyledon

powders. There were 15.30 and 10.12 % adult emergence in the dosage levels of 1.0 and 2.0 g.

Protectant Ability of Maize Grains Treated With Cotyledon Powders and Oil Extracts of *C. Vulgaris*

Maize grains treated with powders of *C. vulgaris* showed significant different ($p>0.05$) in the reduction of damage caused by *S. zeamais* (Tables 5 and 6). The ability of the extracts and powders to reduce grain damage and weight loss increased with increased in concentration. There was neither damage nor weight loss in maize grains treated with 3.0 and 4.0 g of the powders. Also, there was neither damage nor weight loss in maize grains treated with 1.5 and 2.0 % v/w.

Dosage/20g	mean total no of seed	mean of damage	mean % grain damage	% weight loss
1.0	90.3	20.00±1.00b	22.20±0.4b	5.00±0.32a
2.0	88.2	17.22±1.00b	0.00±0.00a	5.00±1.02a
3.0	89.3	0.00±0.00a	0.00±0.00a	0.00±0.00a
4.0	90.3	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.0	88.3	74.25±0.32c	87.12±0.30c	85.02±0.35b

Table 5: Protectantability of *C.vulgaris* cotyledon powders on maize grains.
Means in the same column followed by the same letter(s) are not significantly different.

Conc (%v/w)	mean total no of grains	mean no of damage seeds	mean % grain damage	% weight loss
0.5	90.4	10.0±0.4b	11.12±2.0b	15.34±0.4b
1.0	87.1	8.41±2.24b	9.13±0.3b	10.22±3.1b
1.5	88.2	0.00±0.00a	0.00±0.00a	0.00±0.00a
2.0	89.2	0.00±0.00a	0.00±0.00a	0.00±0.00a
0.0	90.3	78.00±22c	87.5±2.23c	78.32±0.3c

Table 6: Protectantability of *C. vulgaris* cotyledon extracts on maize grains.
Means in the same column followed by the same letter(s) are not significantly different.

Discussion

Effective storage of grains and food products against insect damage is a serious concern [5]. Residual chemical insecticides are currently used around the world to control stored product insects. One of the shortcomings of these conventional insecticides is the conversion of innocuous species to pest and the evolution of resistant forms [19] in addition to environmental pollution, ozone layer depletion, cost and health hazards. These have led to the quest for the use of bioactive pesticides for protection of stored grains to serve as alternatives to the chemical pesticides [5].

The present investigation was undertaken to study weevil mortality, oviposition, adult emergence, and the protectant ability of the cotyledon powders and oil extracts on store maize grains. It was revealed in this study that maize grains treated with cotyledon oil extracts and cotyledon powder of *C. vulgaris* at the concentration of 0.5, 1.0, 1.5, and 2.0 % v/w and powder dosage rate of 1.0, 2.0, 3.0, and 4.0 of per 20 g of maize grain possessed promising level of control of *S. zeamais* in terms of reduction in oviposition, increase in mortality of weevils, reduction of grain damage and weight loss. The mode of action of the oil has been ascribed to physical barrier created that hinder respiration of the insect eggs and growing larvae to respire [20]. It has also been suggested that the mechanical effect of large quantities of powder themselves could have effect on oviposition. In fact, the cotyledon powders and oils of *C. vulgaris* evoked a very high level of mortality of *S. zeamais*, resulting in low oviposition and adult emergence. This mode of action could be attributed to stomach poison since the weevils feed directly on the grains [4,21]. This action could also be attributed to some active ingredients of plant which may possess contact, stomach and respiratory poisoning properties [22]. Plant oils are commonly used in insect control because they are relatively efficacious against virtually all life stages of insects [23,24]. This is in agreement with the result obtained in the present study.

Oil from cotyledon of *C. vulgaris* resulted in higher mortality of *S. zeamais* compared to the cotyledon

powder. This finding is in conformity with the report of Yalamanchilli and Punukollu [25] who observed that the oil from the leaves of *Curcuma domestica* protected the seeds against *Callosobruchus chinensis*, at 2.0% concentration. The result is also in agreement with the findings of Al-Moajel [6] that affirmed the insecticides effect of *Sessbamia sesban* seed oil on *Sitophilus granarium.*

The reduced oviposition and inhibition of adult emergence observe in some cotyledon powders and oil extracts could be as a result of high adult mortality of *S. zeamais*. The oil inhibited locomotion which affects mating activities [22,26-28]. The few eggs that are laid are unable to stick to the surface of the seeds as a result of the oil and this also prevents progeny production. The cotyledon powders and oil completely protected the seeds from been damage by *S. zeamais.* The protectant ability of these powders and oil were highly remarkable. This may be attributed to ovicidal and larvidal properties of the cotyledon of *C. vulgaris* that completely killed some of the eggs that were laid and also prevented the few ones that were hatched into larva from pupating. Thus, the results obtained from this study confirmed that powders and oils of cotyledon of *C. vulgaris* can be used as insecticides against *S. zeamais*.

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